Claims

1. A method for the detection of the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising the steps of
   (a) treating a sample comprising said nucleic acid molecule or consisting of said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in
      (i) methylated form; or
      (ii) non-methylated form
   to pair with a nucleotide normally not pairing with said nucleotide prior to conversion;
   (b) amplifying said nucleic acid molecule treated with said agent;
   (c) real-time sequencing said amplified nucleic acid molecule; and
   (d) detecting whether said nucleotide is methylated or not methylated in said predetermined position in the sample.

2. The method of claim 1 wherein said sample is derived from a tissue, a body fluid or stool.

3. The method of claim 2 wherein said tissue is a tumor tissue, a neurodenerative tissue or a tissue affected with another neurological disorder.

4. The method of any one of claims 1 to 3 wherein said nucleic acid molecule is a DNA molecule or an RNA molecule.

5. The method of any one of claims 1 to 4 wherein the amplification in step (b) is effected by LCR or PCR.

6. The method of claim 5 wherein one amplification primer is detectably labeled.

7. The method of claim 6 wherein said label is biotin, avidin, streptavidin or a derivative or a magnetic bead.

8. The method of any one of claims 1 to 7 wherein said methylated nucleotide is an adenine, guanine or a cytosine.

9. The method of any one of claims 1 to 5 wherein said real-time sequencing comprises:
   (a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form;
   (b) addition of a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine-phosphosulfate (APS) and luciferin;
   (c) sequential addition of all four different dNTPs;
   (d) detection of a luminescent signal wherein the intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule and wherein the intensity of said signal is indicative of the methylation status of said nucleotide in said predetermined position.

10. The method of any one of claims 1 to 9 further comprising quantifying the methylated nucleotides.

11. The method of any one of claims 1 to 10 wherein said agent suitable for the conversion of said nucleotide to pair with a nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bifulfite.

12. A method for the diagnosis of a pathological condition or the predisposition for a pathological condition comprising detection of the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising the steps of
   (a) treating a sample comprising said nucleic acid molecule or consisting of said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in

(i) methylated form; or

(ii) non-methylated form

to pair with a nucleotide normally not pairing with said nucleotide prior to conversion;

(b) amplifying said nucleic acid molecule treated with said agent;

(c) real-time sequencing said amplified nucleic acid molecule; and

(d) detecting whether said nucleotide is methylated or not methylated in said predetermined position in the sample wherein a methylated or a not methylated nucleotide is indicative of a pathological condition or the predisposition for said pathological condition.

13. The method of claim 12 wherein said pathological condition is cancer, a neurodegenerative disease or another neurological disorder.

14. The method of claim 13 wherein said cancer is a primary tumor, a metastasis or a residual tumor.

15. The method of claim 14 wherein said primary tumor is a glioma.

16. The method of claim 15 wherein said glioma is an astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, a pilocytic astrocytoma.

17. The method of claim 13 wherein said neurodegenerative disease is Alzheimer's disease, Parkinson disease, Huntington disease, or Rett-Syndrome.

18. The method of claim 13 wherein said neurological disorder is Prader-Willi-Syndrome, Angelman-Syndrome, Fragile-X-Syndrome, or ATR-X-Syndrome.

19. The method of any one of claims 12 to 18 wherein said nucleic acid molecule is a DNA molecule or an RNA molecule.

20. The method of any one of claims 12 to 19 wherein the amplification in step (b) is effected by LCR or PCR.

21. The method of claim 20 wherein one amplification primer is detectably labeled.

22. The method of claim 21 wherein said label is biotin, avidin, streptavidin or a derivative or a magnetic bead.

23. The method of any one of claims 12 to 22 wherein said methylated nucleotide is an adenine, guanine or a cytosine.

24. The method of any one of claims 12 to 23 wherein said real-time sequencing comprises:

(a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form;

(b) addition of a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine-phosphosulfate (APS) and luciferin;

(c) sequential addition of all four different dNTPs;

(d) detection of a luminescent signal wherein the intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule and wherein the intensity of said signal is indicative of the methylation status of said nucleotide in said predetermined position.

25. The method of any one of claims 12 to 24 further comprising quantifying the methylated nucleotides.

26. The method of any one of claims 12 to 25 wherein said agent suitable for the conversion of said nucleotide to pair with a nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bisulfite..

27. The method of any one of claims 1 to 26 wherein said method is a high-throughput method.